The Annals of Clinical and Analytical Medicine
Original Research

Multidrug resistance-1 gene C3435T single nucleotide polymorphism in OSAS patients

Ceylan Ayada[1], Ümran Toru Erbay[2], Ali Nihat Annakkaya[3]
[1]Department of Physiology, Faculty of Medicine, Kütahya Health Sciences University, Kütahya,
[2]Department of Thoracic Medicine, Faculty of Medicine, Kütahya Health Sciences University, Kütahya,
[3]Department of Thoracic Medicine, Faculty of Medicine, Düzce University, Düzce, Turkey

Abstract

Aim: Obstructive sleep apnea syndrome (OSAS) is characterized by decreased airflow (hypopnea) or complete interruption (apnea) and is characterized by airway narrowing, decreased oxyhemoglobin saturation, hypercapnia, and hyperventilation associated therewith. It is known that genetic factors play role in the pathogenesis of OSAS. Multi-drug resistant-1 (MDR-1) gene products have a role in the pathogenesis of several diseases related to oxidative stress. We aimed to evaluate the frequency of MDR-1 gene C/T polymorphism in patients with OSAS. Material and Method: This study was performed on 43 OSAS and 23 healthy individuals. Blood samples from each individual were collected in tubes with ethylenediaminetetraacetic acid (EDTA). DNA was extracted from the blood samples. The MDR-1 gene polymorphism was detected by polymerase chain reaction (PCR) and enzyme digestion techniques. Results: The frequencies of MDR-1 genotypes were found 27.9% for CC, 41.9% for CT and 30.2% for TT in the OSAS group and 17.4% for CC, 65.2% for CT and 17.4% for TT in the control group. The distribution of MDR-1 gene C alleles was found to be 48.8% in COPD group and 50.0% in the control group; T alleles were found to be 51.2% in OSAS group and 50.0% in the control group. There was no statistically significant difference observed between the groups for genotype and allele frequency of MDR-1 gene ($p>0.05$). Discussion: We believe these results can be useful for large-scale population genetic research considering the frequency of the MDR-1 gene variation in OSAS patients in the Turkish population.

Keywords

Obstructive Sleep Apnea Syndrome (OSAS); Multi-Drug Resistant-1 (MDR-1) Gene; C3435T Single Nucleotide Polymorphism; Turkish Population

DOI: 10.4328/ACAM.6072 Received: 02.11.2018 Accepted: 24.11.2018 Published Online: 03.12.2018 Printed: 01.09.2019 Ann Clin Anal Med 2019;10(5): 602-5
Corresponding Author: Ceylan Ayada, Department of Physiology, Kütahya Health Sciences University, Faculty of Medicine, 43100, Kütahya, Turkey.
GSM: +905056331263 T.: +90 2742652286-1212 F.: +90 2742652285 E-Mail: ceylanayada@gmail.com
ORCID ID: https://orcid.org/0000-0002-6272-4173

Introduction

Obstructive sleep apnea syndrome (OSAS) is a sleep-related respiratory disorder characterized as a decrease in airflow (hypopnea) or a complete cessation (apnea), airway narrowing, decreased oxyhemoglobin saturation, hypercapnia, and hyperventilation associated therewith. It is seen that approximately 10% of middle age population and increases with age to average 50% of the population is affected by OSAS [1, 2]. It is known that OSAS risk is getting high with weight gain. Thus, OSAS prevalence affected by raised global obesity should be updated [3, 4]. OSAS can cause metabolic disorders such as cardiovascular diseases which increase morbidity and mortality risks [5, 6]. There are pieces of evidence that OSAS induces oxidative stress and related diseases [7] but this relation needs to be clarified in details.

Multidrug resistance (MDR) gene belongs to the ATP-binding cassette (ABC) superfamily which constitutes membrane transport systems. These transport systems efflux any kind of cytotoxic material for cell to the outside of the cell [8]. This action is providing the protection of living cells from oxidative stress [9]. If we consider the relationship of oxidative stress, OSAS and MDR-1 gene polymorphism, we believe to know about this polymorphism in OSAS can provide benefits for diagnostic and treatment processes of OSAS. As it has been observed, there is no any study investigated the relationship of OSAS and MDR-1 gene C>T polymorphism. Thus, we aimed to evaluate the frequency of MDR-1 gene C>T polymorphism in patients with OSAS in Turkey.

Material and Method

Patients

This study was performed on 43 OSAS patients who were treated at Düzce University Medical Faculty, Department of Chest Diseases, Düzce (a city located in the Marmara-northern-west region of Turkey) and 23 healthy age- and gender-matched control participants who were obtained from Kütahya Health Sciences University, Medical Faculty, Department of Chest Diseases, Kütahya (a city located in the Aegean part of Turkey) were placed in this study. All of the procedures were explained to the subjects and written informed consent forms were obtained from all participants.

The diagnosis of OSAS was established on the basis of criteria proposed by the guideline establishes clinical practice recommendations for the diagnosis of OSAS in adults. All of the participants were ethnically Caucasian. Individuals who have comorbidities were excluded from the study [10].

Genotyping

Deoxyribonucleic acid isolation

Blood samples of 66 participants (43 OSAS, 23 controls) were collected in tubes with ethylenediaminetetraacetic acid (EDTA). Deoxyribonucleic acid (DNA) samples were isolated from peripheral blood leukocytes by standard phenol/chloroform extraction method.

Polymerase chain reaction

Polymerase chain reaction (PCR) was used to detect C3435T single nucleotide polymorphism (SNP). PCR assay using the forward primer MDR1F 5'-TGC TGG TCC TGA AGT TGA TCT GTG A AC-3' and the reverse primer MDR1R 5'-ACA T TA GGC AGT GAC TCG ATG A AG GCA-3' was performed with 10× buffer, 1.5 mM $MgCl_2$ and 0.2 mM each dNTP and 1 U Taq DNA polymerase [11] .

PCR amplification consisted of an initial denaturation for 2 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature 60°C for 30 s, and extension at 72°C for 30 s. Terminal elongation was performed at 72°C for 4 min. Visualization of PCR product has been presented by Figure 1.

The digestion of a 248-bp PCR product with restriction enzyme MboI for 2 h at 37°C followed this step. Digested products were separated on a 3% agarose gel with ethidium bromide. Subsequently, restriction fragments were identified using the UVI Gel Documentation system. Fragments obtained were 238 bp and 60 bp to T/T genotype, 172 bp and 60 bp fragments to the C/C genotype, and 238 bp, 172 bp and 60 bp to the C/T genotype [12] (Figure 2).

Statistical analysis

Statistical analyses were done by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. All results are given as number and percentage. Statistical significance of the observed genotype frequencies was evaluated ac-

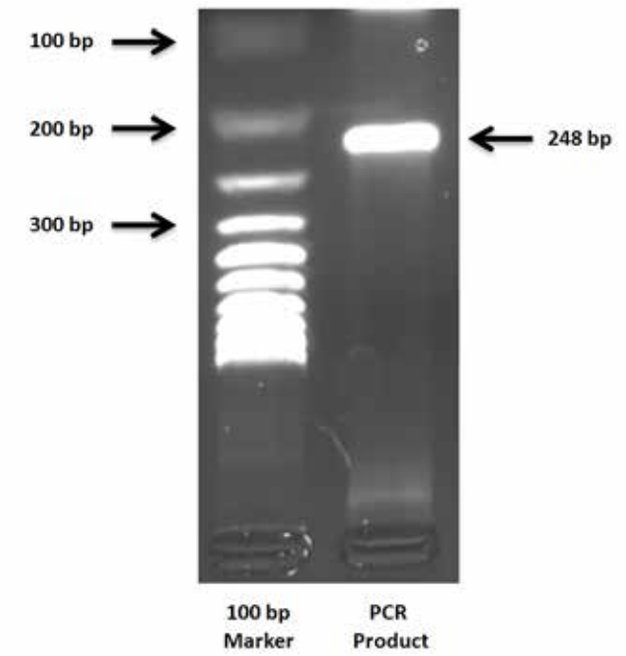

Figure 1. PCR product

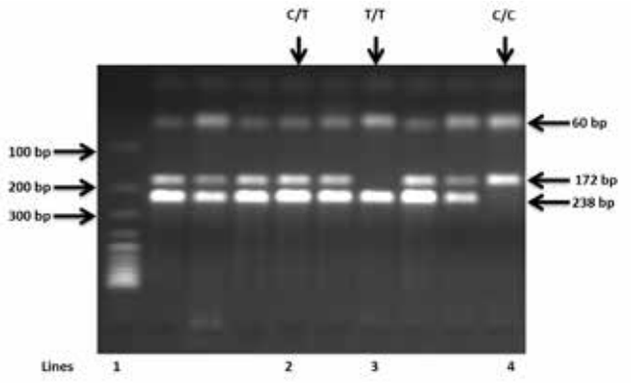

Figure 2. Agarose gel electrophoresis of MDR-1C3435T PCR products digested with MboI restriction enzyme. Line 1: 100 bp DNA Marker; Line 2: heterozygote for C and T allele (238, 172 and 60 bp); Line 3: Homozygote for T allele (238 and 60 bp); Line 4: Homozygote for C allele (172 and 60 bp).

cording to the Hardy-Weinberg rule compared to the expected genotype frequencies. The Chi-square test was used for comparison of genotype and allele frequencies between groups. All p- values < 0.05 were accepted as statistically significant.

Results

The frequency of genotype for MDR gene C/T polymorphism in the OSAS and control groups did not show a significant deviation from the *Hardy-Weinberg* equilibrium (p > 0.05) (Table 1).

The frequencies of MDR genotypes in patients with OSAS and in control subjects are shown in Table 2. The distribution of MDR genotypes were found to be 27.9% (12) for CC, 41.9% (18) for CT, and 30.2% (13) for TT in the OSAS group and 17.4% (4), 65.2% (15), and 17.4% (4) for CC, CT, and TT, respectively, in the control group. There was no statistically significant difference between groups for MDR genotype frequencies (c^2 = 3.278; df = 2; p = 0.194) (Table 2).

The allele frequencies for the MDR gene in the OSAS patients and the control subjects were shown in Table 2. The distribution for the MDR gene C alleles was found to be 48.8% (42) in the OSAS group and 50.0% (23) in the control group; T alleles were found to be 51.2% (44) in the OSAS group and 50.0% (23) in the control group. There was no statistically significant difference between the groups for allele frequency (c^2 = 0.016; df = 1; p = 0.899) (Table 2).

Table 1. Hardy-Weinberg equilibrium of the MDR gene C/T polymorphism

		OSAS		Control	
	Allel	Expected	Observed	Expected	Observed
Common homozygotes	CC	10.4	12	5.6	4
Heterozygotes	CT	21.5	18	11.5	15
Rare homozygotes	TT	11.1	13	5.9	4
		p = 0.285		p = 0.144	

Table 2. Genotype and allele frequencies of the MDR C/T polymorphism

	OSAS		Control	
	n	%	n	%
Genotype Frequency				
MDR C/T polymorphism				
CC	12	27.9	4	17.4
CT	18	41.9	15	65.2
TT	13	30.2	4	17.4
Total	43	100	23	100
c^2 = 3.278; df = 2; p = 0.194				
CC and CT	30	69.8	19	82.6
TT	13	30.2	4	17.4
Total	43	100	23	100
c^2 = 1.292; df = 1; p = 0.256				
CC	12	27.9	4	17.4
CT and TT	31	72.1	19	82.6
Total	43	100	23	100
c^2 = 0.902; df = 2; p = 0.342				
Allele Frequency				
MDR C allele	42	48.8	23	50.0
MDR T allele	44	51.2	23	50.0
c^2 = 0.016; df = 1; p = 0.899				

OSAS = Obstructive sleep apnea; df = degrees of freedom

Discussion

OSAS is known as one of the most general sleep-related breathing health problem. Collapsing of upper airway recurrently causes OSAS development [1, 2]. Intermittent respiratory arrest in OSAS results in recurrent hypoxic and re-oxygenated conditions in the body. This cycle changes the oxidative balance and causes the formation of reactive oxygen radicals [13]. The unbalanced oxidative state in these patients leads to the development of other additional diseases such as local and systemic inflammation and coagulation-fibrinolysis imbalance [5]; cardiovascular diseases [5]; neurocognitive dysfunction [14] fibromyalgia [14].

P-glycoprotein (P-gp) is produced by the expression of the MDR-1 gene. It plays role as an ATP-driven efflux transmembrane protein [16]. P-gp pumps any kind of unfamiliar substance for cell to outside of the cell. Thus, this pump has a protective role against the oxidative stress by releasing the oxidative stress metabolites to the extracellular area [17, 18]. There are at least over than fifty single nucleotide polymorphisms (SNPs) have been reported for the MDR-1 gene associated with P-gp level. These SNPs have been found various in different populations and subject and have importance for pharmacogenomics and pharmacogenetics. One of the SNP is C3435T have been identified to be a risk factor for certain human diseases [19]. It is known that T/T homozygote can cause decreased expression of P-gp compared with C/C homozygotes [20].

In literature, it is suggested that oxidative stress is caused by central obesity in OSAS rather than intermittent hypoxia or respiratory disturbances [21]. In another study presented that increased oxidative stress via glutathione/oxidized glutathione (GSH/GSSG) pathway can provide OSAS development. In this study, investigators excluded co-morbidities and the other factor which can increase oxidative stress [22]. If we consider about controversially results in the literature, we believe that the relationship between oxidative stress and OSAS needs to be clarified at a different points of view.

Some polymorphisms that play a role in the development of OSAS have been reported. At the same time, there are studies describing the necessity of considering polymorphism diversity in the treatment of OSAS [23-25]. As we observed that our data is the first study to evaluate MDR1 gene C3435T SNP in OSAS patients. As a result of Hardy-Weinberg evaluations, we can say that our population expansion has enough numbers to evaluate the polymorphism what we are interested in. For this reason, we believe that the data we have obtained are in the way of contributing literature. In addition, if we consider the role of ethnic factors in terms of polymorphism diversity, the assessment of MDR-1 polymorphism in OSAS patients in different populations may be useful in explaining OSAS pathophysiology and efficacy in treatment. In our study, we aimed to contribute to the pathophysiology and treatment of OSAS as a result of oxidative stress increase caused by reduced P-gp expression in MDR-1 gene C3435T SNP polymorphism T/T homozygous individuals. As a result of the obtained data, there was an increase in T/T homozygous individuals in the OSAS group compared to the control group, but not statistically significant.

If we consider allelic frequencies, OSAS group had a statistically insignificant elevation in T allele compared to C allele. Therefore, we think that oxidative stress that may develop due to P-gp decrease in T/T homozygous individuals may be involved in OSAS pathophysiology. In both OSAS and control groups, the number of heterozygous individuals was more determined com-

pared to other genotypes. For this reason, we can predict that the individuals with comorbidities which is been developed due to T allele are eliminated from the population due to developed complications and shortened lifespan.

Conclusions

Explaining OSAS pathophysiology will certainly provide high added value in effective treatment of the disease. Examination of MDR-1 gene C3435T polymorphism in patients with OSAS may provide opportunities for the development of effective specific therapeutics, as well as providing a more accurate assessment of OSAS pathophysiology in terms of oxidative stress. We, therefore, believe in the necessity of evaluating the relevant polymorphisms in different and enlarged populations in OSAS patients.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Younes M. Role of respiratory control mechanisms in the pathogenesis of obstructive sleep disorders. J Appl Physiol. 2008; 105: 1389–405.
2. Arnold J, Sunilkumar M, Krishna V, Yoganand SP, Kumar MS, Shanmugapriyan D. Obstructive Sleep Apnea. J Pharm Bioallied Sci. 2017; 9: 26–8.
3. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med. 2002; 165: 1217–39.
4. Woods CE, Usher K, Maguire GP. Obstructive sleep apnoea in adult indigenous populations in high-income countries: an integrative review. Sleep Breath. 2015; 19: 45–53.
5. Zamarron C, García Paz V, Riveiro A. Obstructive sleep apnea syndrome is a systemic disease. Current evidence. Eur J Intern Med. 2008; 19: 390–8.
6. Bonsignore MR, Zito A. Metabolic effects of the obstructive sleep apnea syndrome and cardiovascular risk. Arch Physiol Biochem. 2008; 114: 255–60.
7. Eisele HJ, Markart P, Schulz R. Obstructive Sleep Apnea, Oxidative Stress, and Cardiovascular Disease: Evidence from Human Studies. Oxid Med Cell Longev. 2015; 2015: DOI: 10.1155/2015/608438
8. Drach J, Gsur A, Hamilton G, Zhao S, Angerler J, Fiegl M, et al. Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4, and interferon-gamma in normal human T lymphocytes. Blood. 1996; 88(5): 1747–54.
9. Van der Deen M, Marks H, Willemse BW, Postma DS, Müller M, Smit EF, et al. Diminished expression of multidrug resistance-associated protein 1 (MRP1) in bronchial epithelium of COPD patients. Virchows Arch. 2006; 449(6): 682–8.
10. Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med. 2017; 13: 479–504.
11. Turgut S, Turgut G, Atalay EO. Genotype and allele frequency of human multidrug resistance (MDR1) gene C3435T polymorphism in Denizli province of Turkey. Mol Biol Rep. 2006; 33(4): 295–300.
12. Akin M, Turgut S, Ayada C, Polat Y, Balci YI, Erdoğan F. Relation between 3435C 4 T multidrug resistance 1 gene polymorphism with high dose methylprednisolone treatment of childhood acute idiopathic thrombocytopenic purpura. Gene. 2011; 487: 80–3.
13. Lira AB, de Sousa Rodrigues CF. Evaluation of oxidative stress markers in obstructive sleep apnea syndrome and additional antioxidant therapy: a review article. Sleep Breath. 2016; 20: 1155–60.
14. Zhou L, Chen P, Peng Y, Ouyang R. Role of Oxidative Stress in the Neurocognitive Dysfunction of Obstructive Sleep Apnea Syndrome. Oxid Med Cell Longev. 2016; DOI:10.1155/2016/9626831.
15. Yildirim T, Alp R. The role of oxidative stress in the relation between fibromyalgia and obstructive sleep apnea syndrome. Eur Rev Med Pharmacol Sci. 2017; 21: 20–9.
16. Gottesman MM, Hrycyna CA, Schoenlein PV, Germann UA, Pastan I. Genetic analysis of the multidrug transporter. Annu Rev Genet. 1995; 29: 607–49.
17. Schinkel AH. The physiological function of drug-transporting P-glycoproteins. Semin. Cancer Biol. 1997; 8: 161–70.
18. Brambila-Tapia AJ. MDR1 (ABCB1) polymorphisms: functional effects and clinical implications. Rev Invest Clin. 2013; 65: 445–54.
19. Li YH, Wang YH, Li Y, Yang L.Yi MDR1 gene polymorphisms and clinical relevance. Chuan Xue Bao. 2006; 33: 93–104.
20. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A. 2000; 97: 3473–8.
21. Lee SD, Ju G, Choi JA, Kim JW, Yoon IY. The association of oxidative stress with central obesity in obstructive sleep apnea. Sleep Breath. 2012; 16: 511–7.
22. Ntalapascha M, Makris D, Kyparos A, Tsilioni I, Kostikas K, Gourgoulianis K, et al. Oxidative stress in patients with obstructive sleep apnea syndrome. Sleep Breath. 2013; 17:5 49–55.
23. Larkin EK, Patel SR, Goodloe RJ, Li Y, Zhu X, Gray-McGuire C, et al. A candidate gene study of obstructive sleep apnea in European Americans and African Americans. Am J Respir Crit Care Med. 2010; 182: 947–53.
24. Parish JM. Genetic and immunologic aspects of sleep and sleep disorders. Chest. 2013; 143: 1489–99.
25. Patel SR, Goodloe R, De G, Kowgier M, Weng J, Buxbaum SG, et al. Association of genetic loci with sleep apnea in European Americans and African-Americans: the Candidate Gene Association Resource (CARe). PLoS One. 2012; 7(11). DOI: 10.1371/journal

How to cite this article:
Ayada C, Erbay ÜT, Annakkaya AN. Multidrug resistance-1 gene C3435T single nucleotide polymorphism in OSAS patients. Ann Clin Anal Med 2019;10(5): 602-5.